SEQUENCE MATCHING, SIMPLE SEARCHING

PGA Course in Bioinformatics
Tools for Comparative Analysis
July 15, 2002

Outline

- Sequence alignment algorithms
  - Rigorous Optimality:Needleman-Wunsch and Smith-Waterman
  - Rapid, heuristic algorithms
    - BLAST
    - FASTA
    - and their relatives
- Databases and Search Tools

MAJOR SITES WE WILL USE

- http://www.ncbi.nlm.nih.gov/
- http://workbench.sdsc.edu

Needleman Wunsch Algorithm

- Global alignment:: every residue of the two sequences has to participate
- Guaranteed to calculate an Optimal similarity score
- Begin at the beginning of each sequence and go to the end.
- Cannot detect domains

Smith-Waterman Algorithm

- Optimal Local Alignment
- Guaranteed to find all signficant matches to a given query
- Takes the query sequence versus every sequence in the database
- Can be used with arbitrary scoring systems
- **COMPUTATIONALLY EXPENSIVE!!!**

Scoring Matrices

- Relatively simple for DNA-gap penalties or mismatches-can be made to look at Pu/Py
- Protein matches look also at similarity (leu/ileu)

Protein Scoring Matrices

- Chemical similarity: 210 pairs of aa
- Nearness in Genetic Code
- Chemical similarity, e.g., hydrophobicity
- Observed Substitution Schemes

Observed AA Substitution Matrices

- PAM
- BLOSUM

PAM: Point Accepted Mutation

- DAYHOFF et al.
- Observed residue replacement in related proteins
- GLOBAL alignment, closely related
- A model of molecular evolution

1 PAM = average change in 1% of all amino acid possibilities(1% divergence)

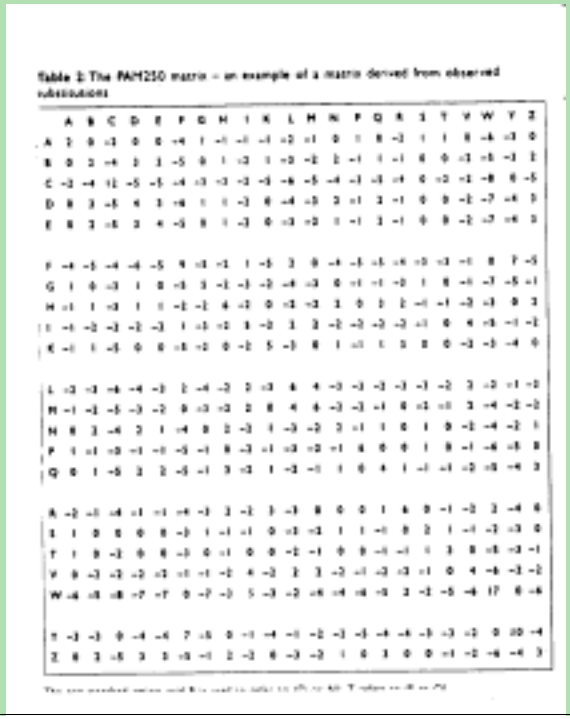
- Other PAM matrices extrapolated from PAM1.

PAM continued

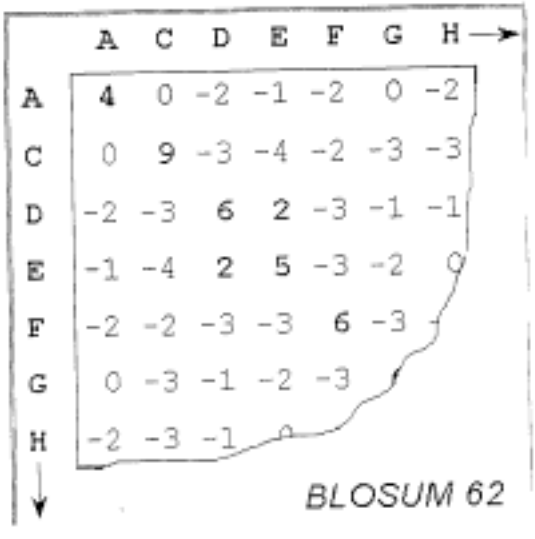
- TIME is NOT correlated with PAM
- Number of the matrix refers to evolutionary distance

Means different families of proteins evolve at different rates

PAM250



Blosum62



BLOSUM

- Block Substitution Matrix
- Henikoff and Henikoff, PNAS, 1992
- Number following indicates per cent identity within set, BLOSUM62=62% id
- Finds short, highly similar sequences (no gaps)

BLOSUM

- Matrices are directly calculated, based on observed alignments
- Greater numbers are lesser distances
- Usually best for local similarity searches
- BLOSUM62= DEFAULT FOR BLAST. If a distant relative, think about another matrix.

BLOSUM SCORING RULES

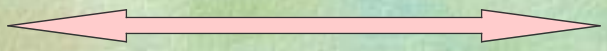
- Zero score means the frequencies of the pair in the database is that expected by chance
- A positive score means more frequent than chance
- Negative score means the pair is found less frequently than chance.

BLAST-**Basic Local Alignment Sequence Tool**

- Objective: find all local regions of similarity distinguishable from random
- Only local alignments permitted,
- Gaps permitted in version 2
- Statistically sound (Karlin and Altschul), but no guantee of optimality

BLOSUM80 BLOSUM62 BLOSUM45

PAM1 PAM120 PAM250



less divergent more divergent

BLAST: Three Step Algorithm

- Compile a list of high scoring words of length w (w=4 for proteins, 12 for nucleic acids)
- Scan for word hits of score greater than threshold, T
- Extend word hit in both directions to find High Scoring Pairs with scores greater than S

Other BLAST Programs

- BLASTN: nucleic acid query to NA database
- BLASTP: Protein query to Protein database
- BLASTX: Translated nucleic acid query to Protein database
- TBLASTN: Protein query against (translated) nucleic acid databse
- TBLASTX: Translated nucleic acid against translated nucleic acid database

OTHER BLAST VARIATIONS

- Gapped BLAST (BLAST 2.0) -extend words from no-gap to gap, generate gapped alignments
- PSI-BLAST- Position Specific Iterated BLAST-use gapped BLAST, generate a Profile from multiple iterations used instead of the input and Distance Matrix

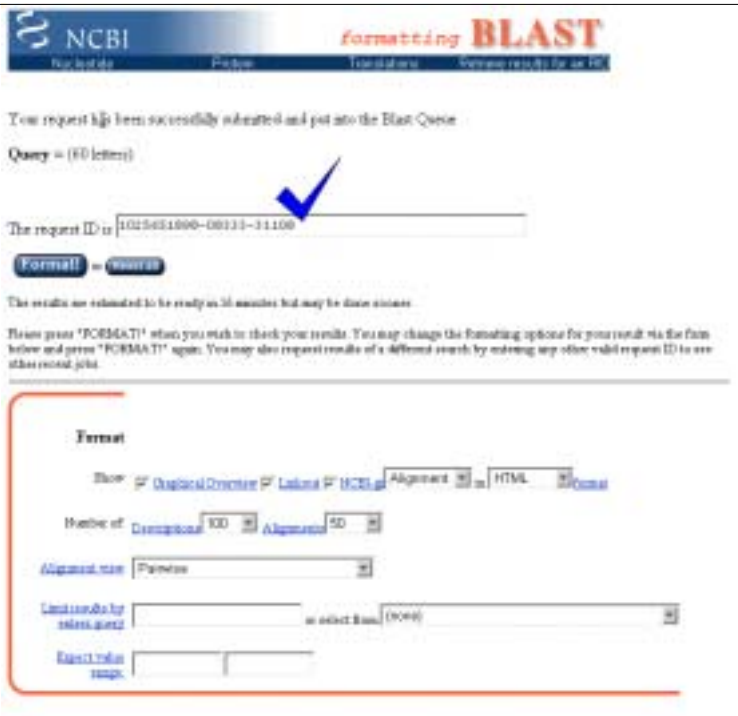
Limitations to BLAST

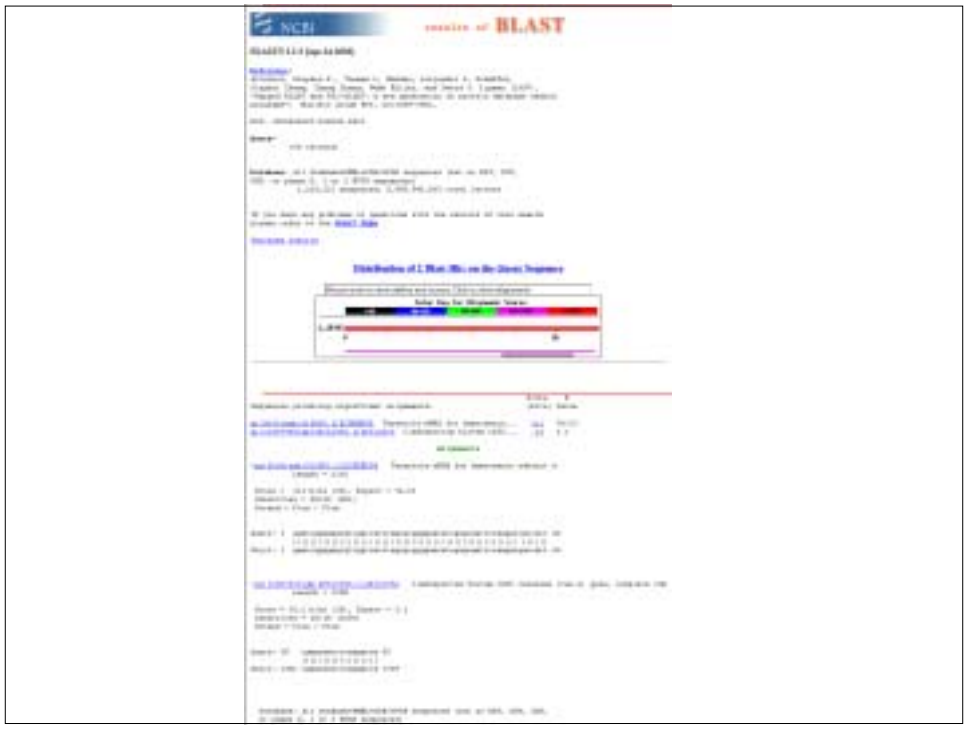
- Needs islands of strong homology
- Limits on the combination of scoring and penalty values
- The variants (blastx, tblastn, tblastx) use 6-frame translation-miss sequences with frameshifts)
- Finds and reports ONLY local alignments

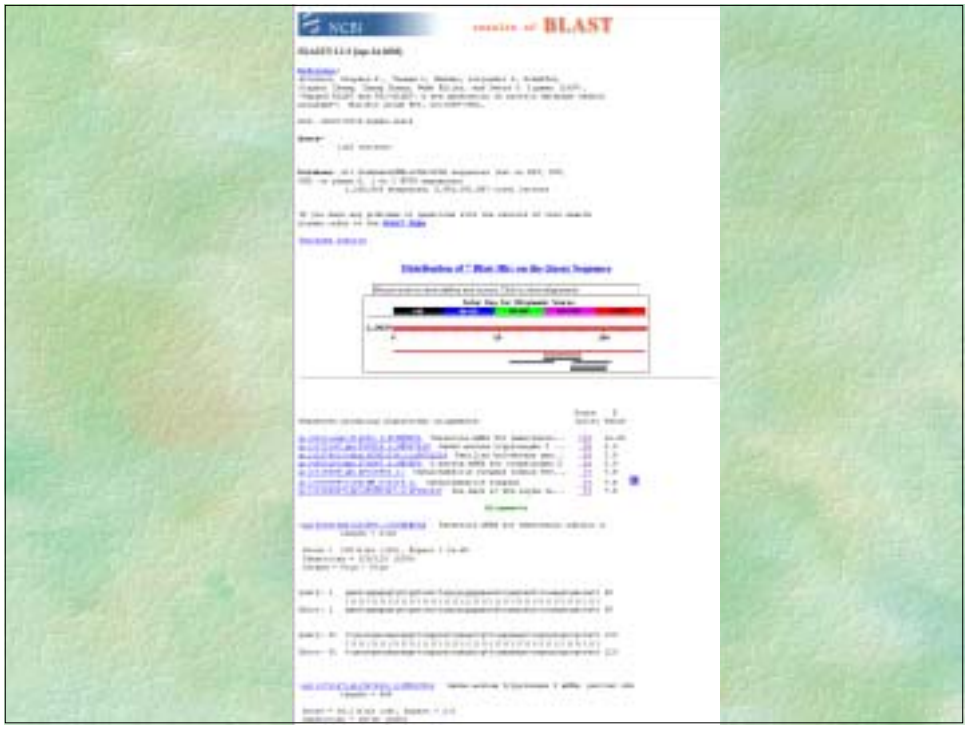
A WALK THROUGH BLAST

NCBI home


What does NCBI do?
Draft Human Genome
BLAST the zebrafish genome!
NCBI in the News


NCBI
formatting BLAST
Your request id has been successfully submitted and put into the Blast Queue
Query = (60 letters)
The request ID is
Format!
Format


NCBI
BLAST


NCBI
BLAST


NCBI
Nucleotide
Search Nucleotide for
Go Clear
Display default Save Text Add to Clipboard

BLAST RULES OF THUMB

- For short amino acid sequences (20-40), 50% identity happens by chance
- If A and B are homologous, and B and C are homologous, then A and C are, even if you can't see it.
- You can get similarity in the absence of homology for low complexity, transmembrane and coiled-coil regions. These have to be eliminated by you.

BLAST Significance

- If you change scoring systems, you can still compare search results if you normalize the score.

  S'=(lambdaS-lnK)/ln2. Lambda and K are associated with the scoring system.

  S', with a given E, is significant if it is greater than log N/E, N the size of the search space.

FASTA: FAST Alignment

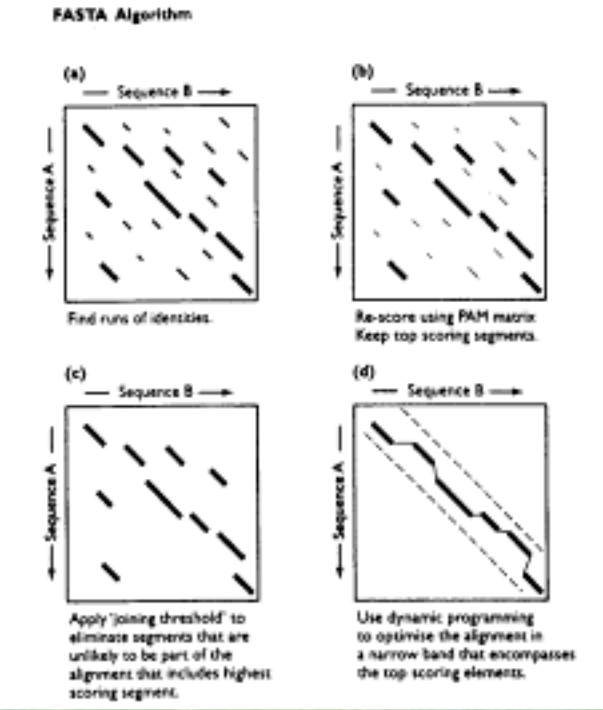
- http://alpha10.bioch.virginia.edu/fasta/
- http://www2.ebi.ac.uk/fasta3
- http://workbench.sdsc.edu

- Rapid Global alignment

- Not a strong mathematical basis

FASTA: WHY USE IT?

- Allow alignments to shift frames



LALIGN

- Essentially a FASTA derivative for local alignments
- Compares two proteins to identify regions of similarity
- Will report several sequence alignments within a given sequence
- Works for internal repeats that are missed by FASTA because of gaps.

SITEs for LALIGN

- http://fasta.bioch.virginia.edu/fasta/lalign.htm
- http://xylian.igh.cnrs.fr/bin/lalign-guess.cgi
- http://biowb.sdsc.edu (registration necessary but painless)
- PALIGN http://fasta.bioch.virginia.edu/fasta/palign.htm (plots a graph of the areas of alignment)

ENTREZ: Linked Databases

http://www.ncbi.nlm.nih.gov/Entrez/

- Concept of Neighbor-usually BLAST relationship
- Precomputed=Fast
- Related sequence, structure neighbors, related articles

EST DATABASES:Quality issues

- SEQUENCE QUALITY
  - calculated error less than 1% (Phred-20) is the rule
  - frameshifts and stops common
  - Rules are usually observed by exception
  - There are lots of exceptions in the public data
  - Many 3' UTRs

EST Databases: Quality #2

- CLONE QUALITY
  - Over-representation
  - Tissue specificity
  - Developmental stage specificty
  - Unprocessed mRNA clones
  - Chimeras
  - Contamination

EST Cluster Databases

- STACK-at SANBI http://sanbi.ac.za
- TIGR-animals, plants, other http://www.tigr.org/tdb/tgi.shtml
- Unigene-NCBI
  - Human, mouse, rat, cow,zebrafish
  - mRNAs
  - predicted mRNAs

UNIGENE

- A LIST OF LISTS
  - The cluster and known EST, mRNA pieces
  - Additional annotaion-gene name, etc.
  - Distributed as a subset of dbest

NOT included in the BLAST searchable DB at NCBI

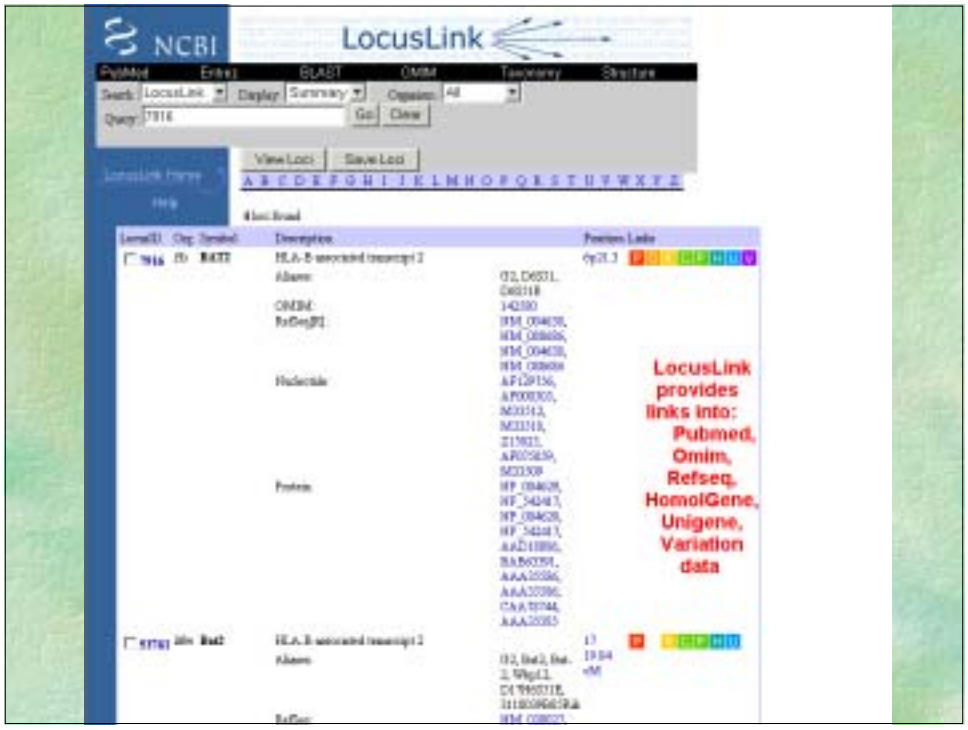
Caveats on Clusters

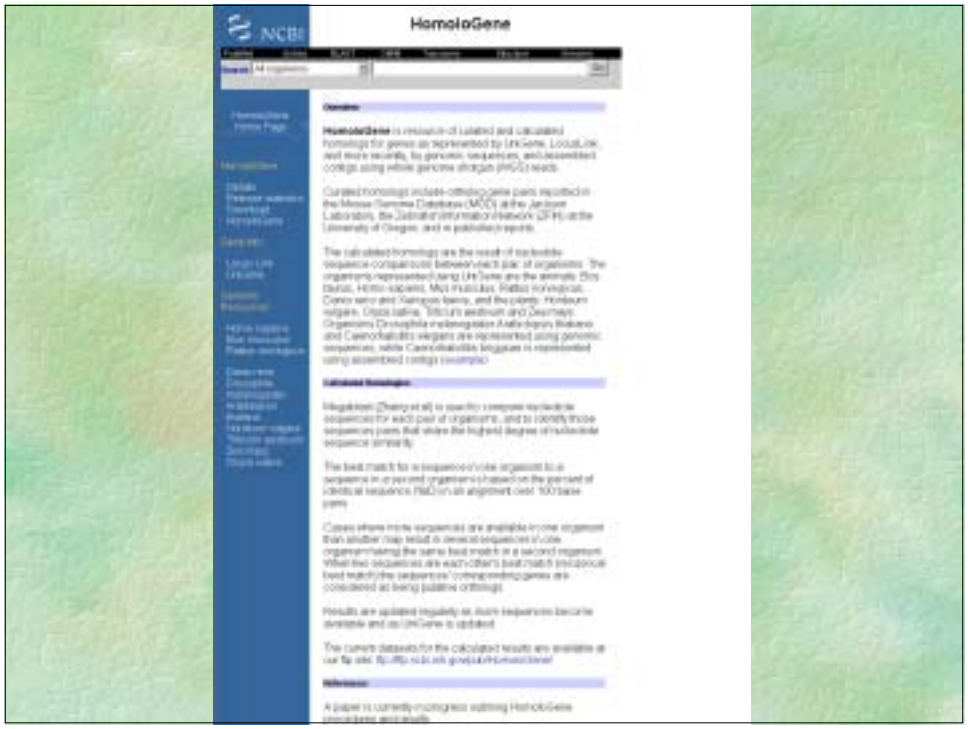
- Not stable
- Can go to complete cDNAs as available

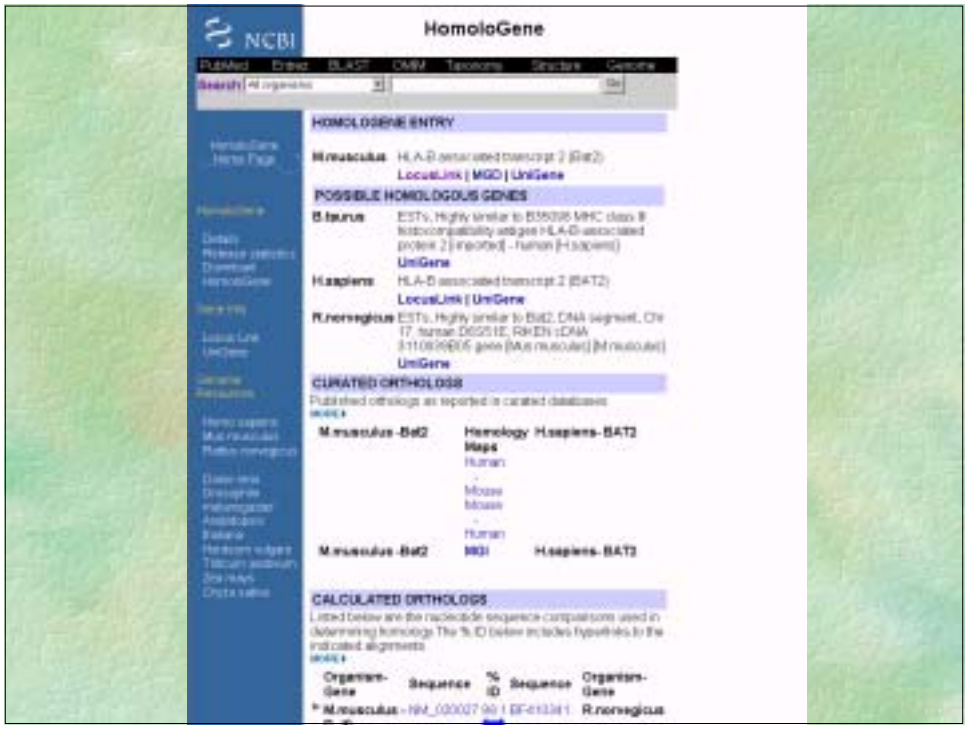
LOCUSLINK

(http://www.ncbi.nlm.nih.gov/LocusLink)

- A useful, searchable compendium of loci across human, mouse, rat, Drosophila and zebrafish
- Linked for PubMed, OMIM, RefSeq, Homologene data, Unigene, and Variation Data


NCBI
LocusLink
LocusLink provides links into: Pubmed, Omim, Refseq, HomoloGene, Unigene, Variation data


NCBI
HomoloGene



Resources for Genomic Comparison

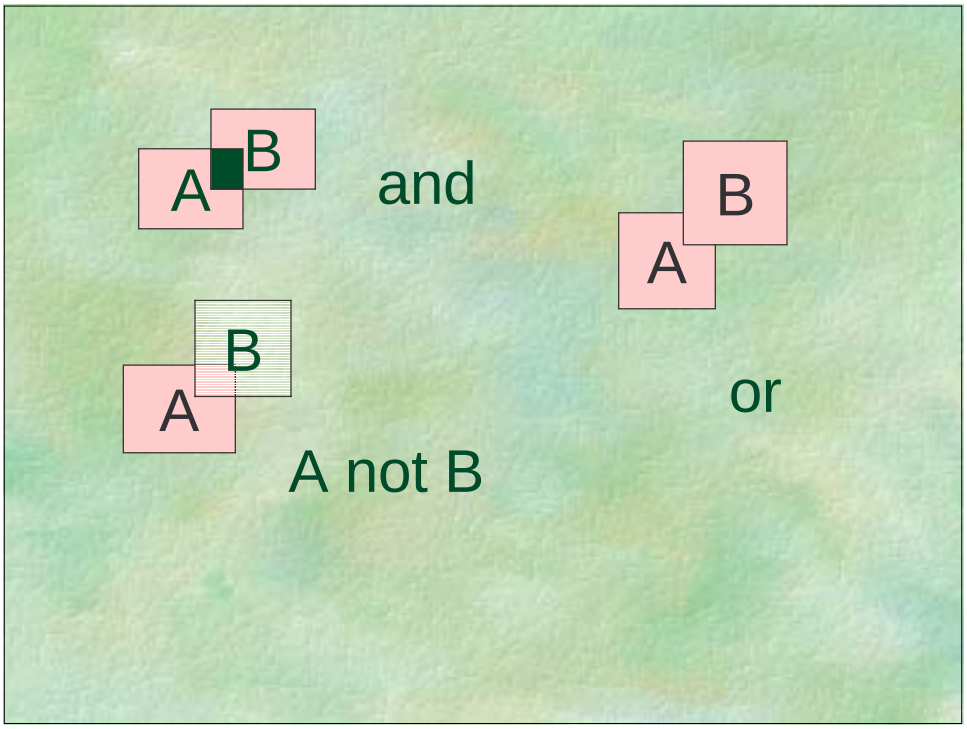
- GLASS-http://plover.lcs.mit.edu
- PipMaker: http://bio.cse.psu.edu
- Rosetta: http:// plover.lcs.mit.edu(genes)
- SGP: htttp://soft.ice.mpg.de/sgp-1
- VISTA: http://www-gsd.lbl.gov/VISTA
- WABA: http://www.cse.ucsc.edu/~kent/xenoAli/index.html

EFFICIENT SEARCHING

- Use Wild Cards: #,$,?,*
- Use Boolean Operators
  - Not
  - And
  - Or
  - Nor

Boolean Operators

- *AND* A and B BOTH
- *OR* A or B EITHER
- *NOT* B not A Have B, do not have A
- *NOR* A nor B A but not B OR B but not A



WILD CARDS

- Match one character-NCBI uses #
- Match zero or one character NCBI uses $, others ?
- Match zero or more characters-usually *

RULES OF THUMB

- Use an up-to-date database; repeat often
- Choose a fast algorithm
- Use the most recent version
- Work at the protein level--for a small amount of evolutionary change, DNA sequence contains less information about homology
- Respect your own ***intuition***

Other Resources

- NCBI Education Page http://www.ncbi.nlm.nih.gov/Education/index.html
- BCM Gene Finder http://searchlauncher.bcm.tmc.edu/docs/sl_links.html
- EBI-SwissProt, TrEMBL, PIR, SRS, Tools http://www.ebi.ac.uk
- ExPASy-SwissProt, TrEMBL http://www.expasy.ch/
- DISC-DNA Information and Stock Center http://www.dna.affrc.go.jp

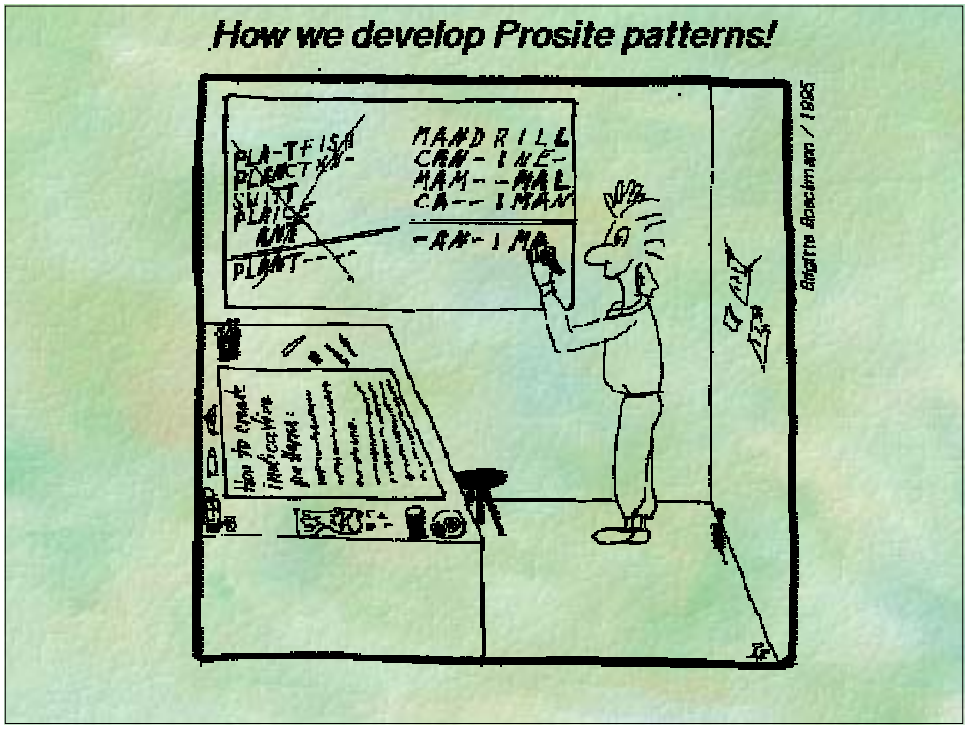
MEDICAL SUBJECT HEADINGS

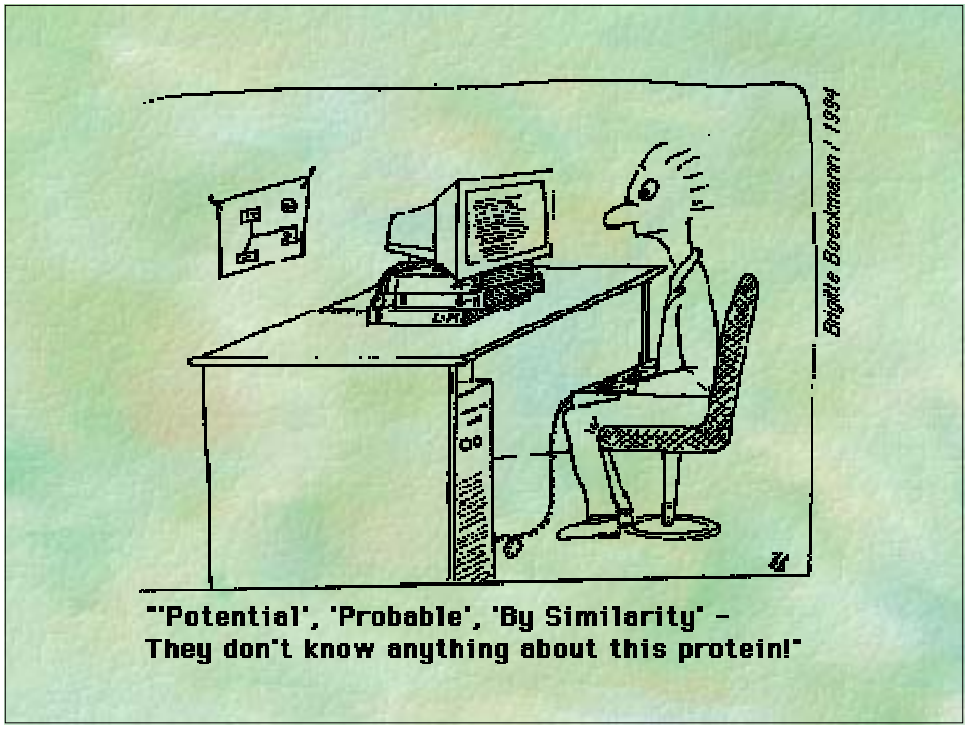
- CONTROLLED Vocabulary
- Indexing of articles, books, etc.
- Current version has over 300,000 terms
- Can download list and make your own assortment

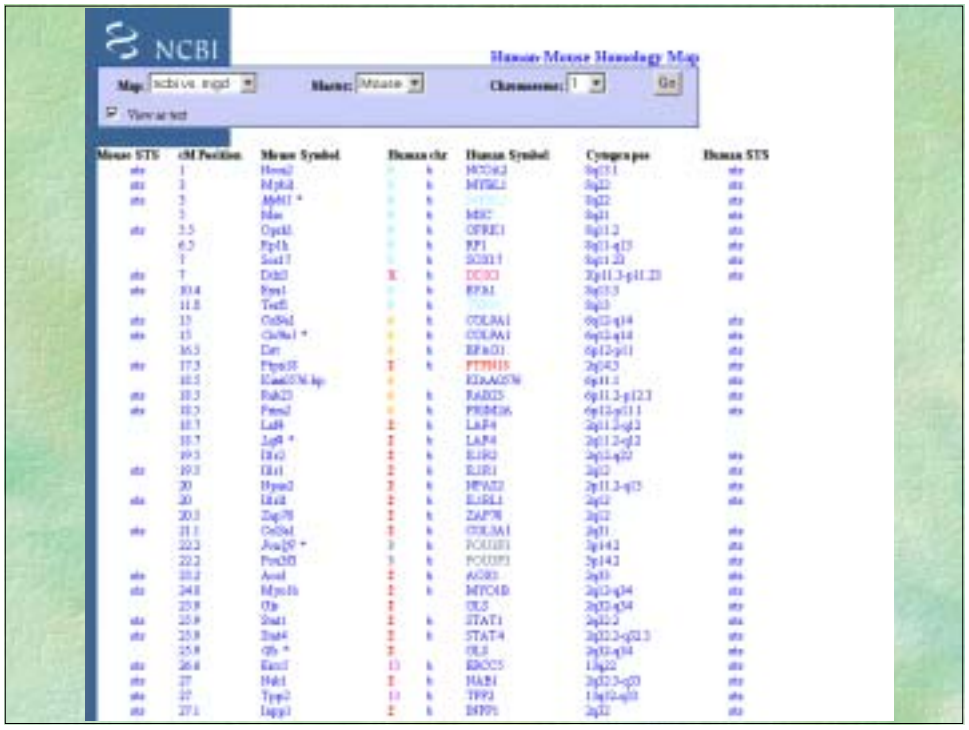
MeSH Advantages

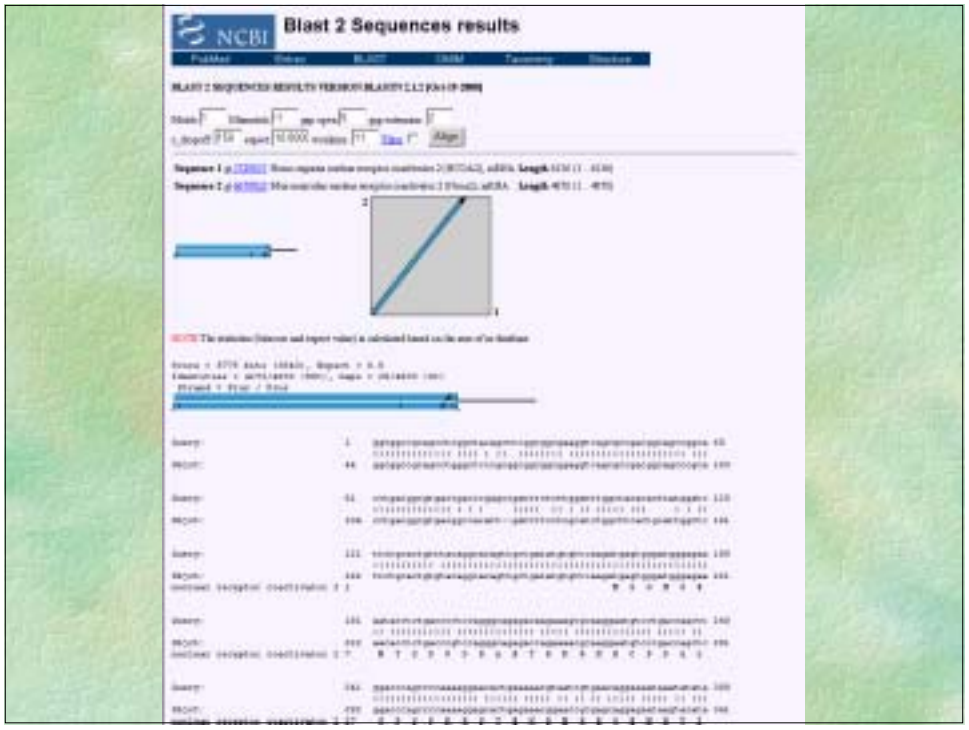
- Assigned to the the entire document, not just title and abstract
- Major topic (*)
- Subheadings if available
- MeSH topics are exploded to include all the terms included in the meaning.

Try it; you may like it.


How we develop Prosite patterns!
MAND R I L L
CAN- I NE-
MAM- -MAL
CA- - I MAN
-AN- I MA
PLANT- -
Brigitte Boeckmann / 1995


Brigitte Boeckmann / 1994
"'Potential', 'Probable', 'By Similarity' -
They don't know anything about this protein!"


NCBI
Human-Mouse Homology Map
Map:
Mouse:
Chromosome:
Go
View as text
Mouse STS
cM Position
Mouse Symbol
Human chr
Human Symbol
Cytogen pos
Human STS


NCBI
Blast 2 Sequences results